Supporting Information

Dupressoir et al. 10.1073/pnas.0902925106

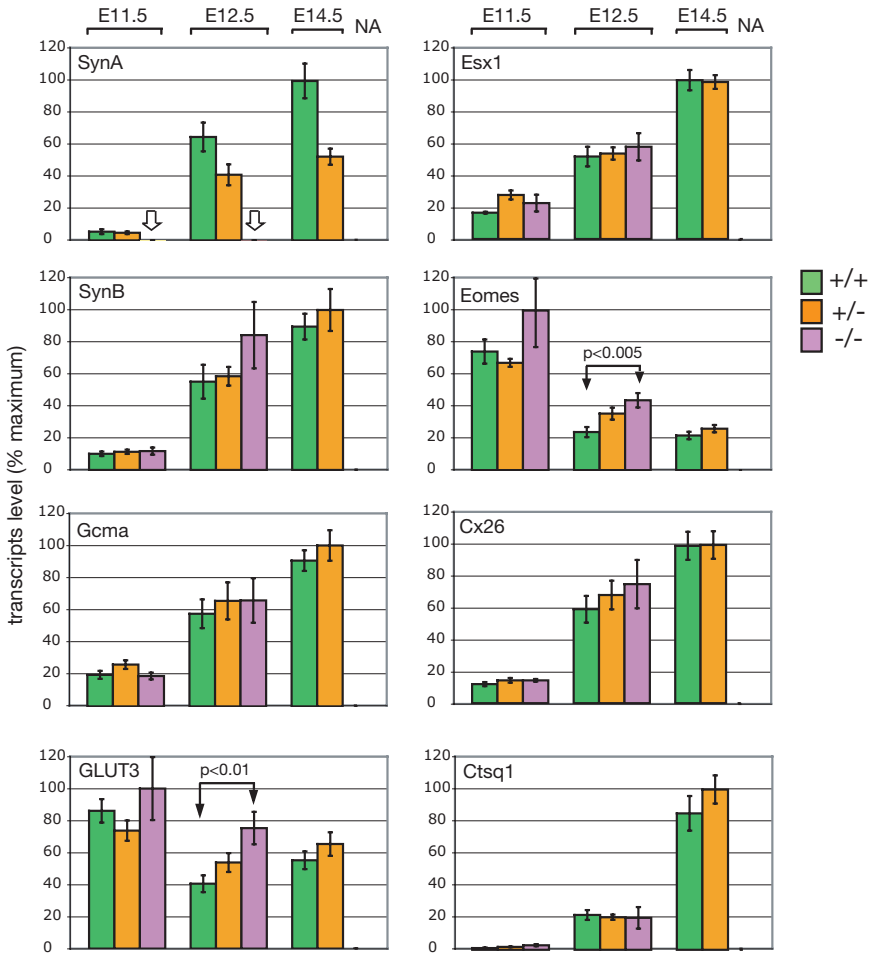

Fig. S1. Real-time quantitative RT-PCR analysis of labyrinthine trophoblast-specific genes in the placentae from wild-type, SynA$^{+/-}$, and SynA$^{-/-}$ living embryos at different embryonic stages (E). Total RNA was extracted from placentae by using the RNeasy RNA isolation kit (Qiagen). Reverse transcription and quantitative PCR were performed as in Dupressoir et al. [Dupressoir A, et al. (2005) *Proc Natl Acad Sci USA* 102:725–730], with primers listed in Table S1. Values (transcript level expressed as percent of maximum, after normalization with 18S RNA) are means ± SEM of 4 to 10 living embryos. White arrows indicate a value below the level of detection, and NA (not applicable) indicates the absence of living E14.5 SynA mutant embryos. Only values that differ significantly (i.e., $P < 0.05$; *t* test) between wild type and mutant are indicated. Genes analyzed are *syncytin-A* [Dupressoir A, et al. (2005) *Proc Natl Acad Sci USA* 102:725–730], *Esx1* [Li Y, Behringer RR (1998) *Nat Genet* 20:309–311], *GCMa* [Anson-Cartwright L, et al. (2000) *Nat Genet* 25:311–314; Schreiber J, et al. (2000) *Mol Cell Biol* 20:2466–2474], and *syncytin-B* [Dupressoir A, et al. (2005) *Proc Natl Acad Sci USA* 102:725–730] {the two latter specific for the syncytiotrophoblast layer ST-II [Simmons DG, et al. (2008) *Development* 135:2083–2091]}; *GLUT3* [Shin BC, et al. (1997) *Endocrinology* 138:3997–4004]; *connexin 26* (*CX26*) [Gabriel HD, et al. (1998) *J Cell Biol* 140:1453–1461]; *Ctsq1* {specific for the sinusoidal trophoblastic giant cells, STGC [Simmons DG, Fortier AL, Cross JC (2007) *Dev Biol* 304:567–578]}; and *mEomesodermin* (*Eomes*) specific of trophoblast stem cells [Tanaka S, et al. (1998) *Science* 282:2072–2075; Russ AP, et al. (2000) *Nature* 404:95–99]. No significant differences between wild-type and mutant placentae were observed, except at E12.5 for the GLUT3 glucose transporter (1.8-fold increase; $P < 0.01$) and mEomesodermin (1.8-fold increase; $P < 0.005$). As expected, no syncytin-A expression was detected in the placentae of null embryos.

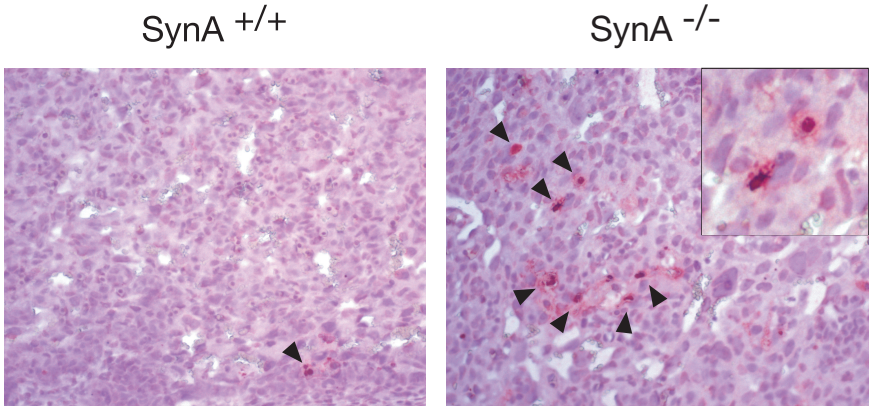

Fig. S2. Increase of apoptotic cell number in the labyrinth of SynA-null placenta. TUNEL assays were performed on 4-μm paraffin sections from E13.5 wild-type and mutant (SynA$^{-/-}$) placentae by using the In Situ Cell Death detection kit (Roche) according to the manufacturer's instructions. Arrows point to TUNEL-positive cells. (*Inset*) Higher magnification of 2 positive cells, with the nucleus stained. Sections of 2 placentae were counted for TUNEL-positive and total cells. Mean percentage of TUNEL-positive cells $\pm$ SEM are: 0.21% $\pm$ 0.07% for SynA$^{+/+}$ and 3.2% $\pm$ 0.4% for SynA$^{-/-}$ ($P < 0.0001$; 2-tailed *t* test). (Original magnification, 200$\times$; inset, 400$\times$.)

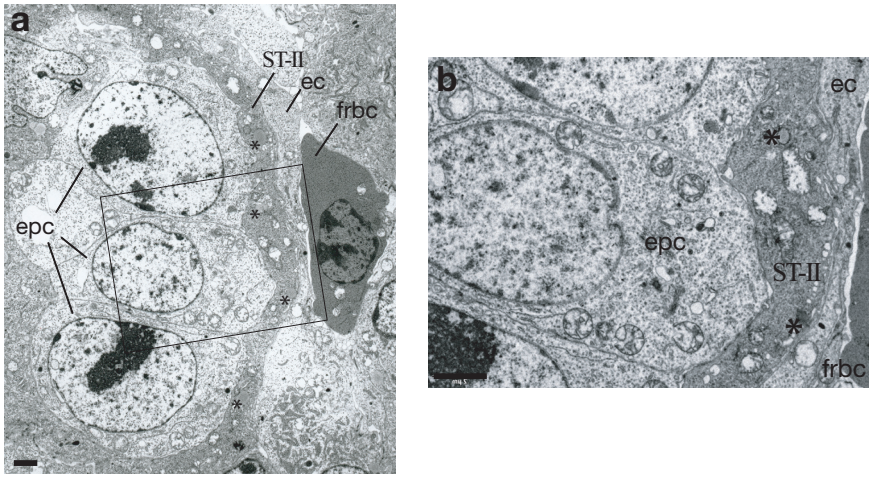

Fig. S3. Electron micrographs of the labyrinth of E11.5 SynA null placentae in a region where the interhemal barrier is not formed but discloses normal development of the ST-II syncytiotrophoblast layer (*a*; expanded view in *b*). The ST-II layer (with typical lipid inclusions, asterisks) interacts—with membrane boundaries of normal appearance—with the endothelial cells (ec) of the fetal blood vessels (frbc, fetal red blood cells), and on the other side with undifferentiated mononuclear trophoblast cells arranged as a parenchyma. The latter are most probably ectoplacental cells (epc), as can be inferred from their large nuclei and lack of lipid vesicles [Hernandez-Verdun D (1974) *Cell Tissue Res* 148:381–396], and are presumed to finally differentiate into the ST-I syncytiotrophoblast layer in normal placenta. (Scale bar: 2 μm.)

Table S1. Oligonucleotides

Name	Sequence
Gene targeting: 5′ and 3′ arm amplification	
5′ arm-F	CCCTTTTTCACTTCCTGCTCCAC
5′ arm-R	TGTTAGGGTCTGCCCCACTTTT
3′ arm-F	TCCATTTTGCTTAAACGGTGGT
3′ arm-R	AGGTCTCACTCAGCGTGGTGGT
Gene targeting: 1.8-kb syncytin-A ORF amplification	
ORF-F	ACTAGCCCCTGATGACCCTCCCC
ORF-R	TGGCAAGGCTGTGGCTAACACG
RT-PCR quantification	
SynA-F	CATCTATGCTGGATGAAGCCT
SynA-R	AGACCCTGGCATGGCCATTA
SynB-F	GCCCGTTGATCTCAGCCTCCT
SynB-R	GGCATCCGGTCTTTTCATTGC
Gcma-F	CTGCCTCCAACTCCTTACGG
Gcma-R	CTGGGAGAGAGAAGGGGAGC
GLUT3-F	CTGAAGAACTGTGGCCTGG
GLUT3-R	GCTTCTCCTGTGACATCCGA
Esx1-F	AGCAACCCCAACAGGAGC
Esx1-R	GGACTCATGGCGACTGGA
Eomes-F	AAAACTTCTTCCCGGAGCC
Eomes-R	TGTCTAGCTTGTTGGTCACAGG
Cx26-F	GCTTCAGACCTGCTCCTTAC
Cx26-R	ATCTCCCCACACCTCCTTT
Ctsq-F	TTCATTGGCCCAATACCCTA
Ctsq-R	GAAAGCTCCCAGAATTCACA